Mechanisms of the Ba^{2+}-induced Contraction in Smooth Muscle Cells of the Rabbit Mesenteric Artery

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ABSTRACT   The mechanism of the Ba^{2+}-induced contraction was investigated using intact and saponin-treated skinned smooth muscle (skinned muscle) strips of the rabbit mesenteric artery. After depletion of Ca^{2+} stored in the caffeine-sensitive site, >0.65 mM Ba^{2+} evoked contraction in muscle strips depolarized with 128 mM K^+ in Ca^{2+}-free solution in a dose-dependent fashion, and the ED_{50} values for Ca^{2+} and Ba^{2+} were 0.5 mM and 1.2 mM in intact muscle strips, respectively. Nisoldipine (10 nM) blocked the contraction evoked by high K^+ or 10 μM norepinephrine (NE) in the presence of 2.6 mM Ba^{2+}, but did not block the contraction evoked in the presence of 2.6 mM Ca^{2+}. These results may indicate that Ba^{2+} permeates the voltage-dependent Ca^{2+} channel. In skinned muscle strips, the ED_{50} values for Ca^{2+} and Ba^{2+} were 0.34 and 90 μM, respectively, as estimated from the pCa- and pBa-tension relationships. Calmodulin enhanced and trifluoperazine inhibited the Ba^{2+}- and Ca^{2+}-induced contractions. After the application of Ba^{2+} or Ca^{2+} with $ATP_\gamma S$ in rigor solution, myosin light chain (MLC) was irreversibly thiophosphorylated, as estimated from the Ba^{2+}- or Ca^{2+}-independent contraction. Furthermore, both divalent cations phosphorylated MLC, as measured using two-dimensional gel electrophoresis, to the extent expected from the amplitudes of the contraction evoked by these cations. Thus, Ba^{2+} is capable of activating the contractile proteins as Ca^{2+} does. The amount of Ca^{2+} or Ba^{2+} stored in cells was estimated from the caffeine response evoked in Ca^{2+}-free solution in intact and skinned muscle strips. After the application of 0.3 μM Ca^{2+} or 0.1 mM Ba^{2+} for 60 s to skinned muscle strips after the depletion of Ca^{2+} stored in cells, caffeine produced a contraction only upon pretreatment with Ca^{2+} but not with Ba^{2+}. When Ba^{2+} was applied successively just after the application of Ca^{2+}, the subsequently evoked caffeine-induced contraction was much smaller than that evoked by pretreatment with Ca^{2+} alone. The above results indicate that (a) Ba^{2+} permeates the voltage-dependent Ca^{2+} channel but may not permeate the receptor-operated Ca^{2+} channel, (b) it releases Ca^{2+} from store sites but is not accumu-

lated into the store site, and (c) it directly activates the contractile proteins via formation of a Ba^{2+}-calmodulin complex.

INTRODUCTION

Barium salts are useful tools for the study of properties of the Ca^{2+} channel at the plasma membrane in various excitable tissues (Hagiwara and Byerly, 1981) and mechanical properties of the contractile proteins (Ebashi and Endo, 1968; Saeki et al., 1984). Ba^{2+} carries the inward current through Ca^{2+} channels in cardiac muscle cells (Lee and Tsien, 1983; Reuter, 1983), as well as in smooth muscle cells (Benham et al., 1985; Inomata and Kao, 1985). The mechanism of the Ba^{2+}-induced contraction in smooth muscles, however, is still controversial. Ba^{2+} may act by increasing the influx of extracellular Ca^{2+} into smooth muscle cells of guinea pig taenia coli to produce the contraction (Antonio et al., 1973; Nasu and Urakawa, 1973), but other studies have indicated that Ba^{2+} contraction is due to the activation of release of Ca^{2+} from the intracellular store site in vascular smooth muscle cells (Northover, 1968; Uvelius et al., 1974; Somlyo et al., 1974). Furthermore, Hansen et al. (1984) suggested that Ba^{2+} may also act directly on the contractile machinery of vascular smooth muscles. Therefore, the present study is intended to clarify the mechanism of the Ba^{2+}-induced contraction in vascular smooth muscle.

Bolton (1979) suggested that Ca^{2+} influx, activated by agonists, is due to activation of the voltage-dependent and the receptor-operated Ca^{2+} channels. The latter produces contraction with or without membrane depolarization by releasing Ca^{2+} from the cellular store site (Somlyo and Somlyo, 1968; Casteels et al., 1977; Ito et al., 1979). However, there is no evidence that Ba^{2+} carries inward current during activation of the receptor-operated Ca^{2+} channel.

It has been demonstrated in smooth muscle tissues that the sarcoplasmic reticulum (SR) plays an essential role in the intracellular Ca^{2+} regulation of the contraction-relaxation cycle, as it does in skeletal and cardiac muscle (Endo, 1977; Bond et al., 1984; Fabiato, 1985; Itoh et al., 1981, 1983, 1985). Somlyo and Somlyo (1971) stated that Ba^{2+} may not be accumulated into the SR. It is of interest to investigate the effects of Ba^{2+} on the Ca^{2+} mobilization regulated by the SR.

Ba^{2+}-induced contractions were recorded in intact and saponin-treated, chemically skinned smooth muscle (skinned muscle) strips of the rabbit mesenteric artery. For investigation of Ba^{2+} action on the Ca^{2+} store site, the caffeine-induced contraction was evoked in intact and skinned muscle strips to estimate the amount of Ca^{2+} or Ba^{2+} stored in cells. To observe the actions of Ba^{2+} on the contractile mechanism in comparison with those of Ca^{2+}, phosphorylation of the myosin light chain (MLC) and the effects of calmodulin or trifluoperazine (TFP), a calmodulin antagonist, on the Ba^{2+}-induced contraction in skinned muscle strips were investigated.

METHODS

Muscle Preparation

Male albino rabbits (1.8–2.2 kg) were anesthetized by sodium pentobarbitone (40 mg/kg i.v.) and exsanguinated. The mesenteric artery of the iliac region was isolated and

transferred to a dissecting chamber filled with Krebs solution (at room temperature). A small circular muscle strip (0.3–0.5 mm in length, 0.05–0.08 mm in width, and 0.02–0.03 mm in thickness) was prepared under a binocular microscope using fine forceps and small knives made from pieces of razor blades.

Experimental Procedures

To measure mechanical activity from intact and skinned muscle strips, the preparation was transferred to a chamber with a capacity of 1 ml; one end of the strip was fixed to the chamber and the other end to a strain gauge (U-gauge, Shinko Co. Ltd., Tokyo). The solution was changed by flushing one end with a syringe and sucking simultaneously from the other end with a water pump.

Mechanical responses evoked by high external K^+ solution in intact muscle strips were measured in the presence of 0.1 μM tetrodotoxin (TTX) and 3 μM guanethidine to prevent the release of norepinephrine (NE) from depolarized nerve terminals.

To prepare skinned muscle strips, the strip was treated with 25 μg/ml saponin in relaxing solution for 20 min (Itoh et al., 1981). To obtain the pCa- or pBa-tension relationship, various concentrations of Ca^{2+} or Ba^{2+} were applied cumulatively. The slopes of the curves are shown as Hill coefficients, n, and the midpoint position, pK ($-\log K$), where K is the dissociation constant. These parameters were obtained by fitting the data points for each curve to the following equation with a nonlinear least-squares method: $P/P_o = (C/K)^n/[1 + (C/K)^n]$, where C represents the concentration of Ca^{2+} or Ba^{2+}, and P/P_o is the normalized tension relative to the contraction evoked by 10 μM Ca^{2+}. As previously reported (Itoh et al., 1986), the contractions induced by repetitive applications of Ca^{2+} deteriorated to a considerable extent (for example, the amplitude of the contraction induced by the third application of 10 μM Ca^{2+} was 0.75 ± 0.05 times that of the first trial [$n = 5$]) and the Ca^{2+} sensitivity of the contractile proteins became lower, but the addition of 0.1 μM calmodulin prevented the deterioration and preserved the Ca^{2+} sensitivity. Therefore, we added 0.1 μM calmodulin to the bath throughout the experiment.

The amount of Ca^{2+} stored in cells was estimated from the amplitude of the contraction evoked by 25 mM caffeine in Ca^{2+}-free solution. However, this method may not introduce the absolute amount of Ca^{2+} stored in cells, because all the Ca^{2+} released from store site may not solely activate the contractile proteins, and the Ca^{2+} stored in cells may be away from the myofilaments. This procedure has been commonly used in skeletal muscles (Endo, 1977), and the results obtained in vascular tissues using this procedure have correlated closely with the results obtained by ⁴⁵Ca experiments (Saida and van Breemen, 1984; Ueno, 1985). During recordings of the caffeine- or A23187-induced contraction, 5 mM NaN_3 or 5 μg/ml oligomycin was added to the solution throughout the experiments to prevent Ca^{2+} mobilization at the mitochondria.

Solutions

The Krebs solution contained (mM): 137.5 Na^+, 5.9 K^+, 134.4 Cl^-, 1.2 Mg^{2+}, 2.6 Ca^{2+}, 15.5 HCO_3^-, 1.2 $H_2PO_4^-$, 11.5 glucose, pH 7.4, bubbled with 97% O_2 and 3% CO_2 mixed gas.

In some experiments, 2.6 mM $CaCl_2$ was replaced with 2.6 mM $BaCl_2$. High external K^+ solution was prepared by replacing NaCl with equimolar KCl. Ca^{2+}-free solution was prepared by substituting $MgCl_2$ for $CaCl_2$ in Krebs solution and adding 1 mM EGTA.

For the experiments on skinned muscle strips, the following solutions were used; relaxing solution was composed of (mM): 110 K-methanesulfonate (KMs), 5 $Mg(Ms)_2$, 5 Na_2ATP, 4 EGTA, 20 PIPES [piperazine-N-N'-bis-(2-ethanesulfonic acid)], and the pH was adjusted to 7.1 with KOH at 25 °C. Solutions of the desired Ca^{2+} or Ba^{2+} concentration

were prepared by adding appropriate amounts of $Ca(Ms)_2$ or $Ba(Ms)_2$ to the relaxing solution. The binding constants of $CaEGTA^{2-}$ and $BaEGTA^{2-}$ were considered to be 4.3 × 10^{10} M^{-1} (at 0.2 M ionic strength; Iino, 1981) and 2.57 × 10^8 M^{-1} (Schwarzenbach et al., 1957), respectively. The binding constants of $CaATP^{2-}$ and $BaATP^{2-}$ were considered to be 4.0 × 10^3 M^{-1} (Martell and Schwarzenbach, 1956) and 1.95 × 10^3 M^{-1} (Taqui Khan and Martell, 1962), respectively. The other binding constants are quoted from the values reported by Iino (1981). A rigor solution was prepared by omitting ATP from the relaxing solution and the free Mg^{2+} concentration was kept at 1 mM. The composition of the $ATP_\gamma S$-containing solution (with 10 μM Ca^{2+} or 1 mM Ba^{2+}) was (mM): 123 KMs, 2.6 $Mg(Ms)_2$, 2 $ATP_\gamma S$, 4 EGTA, 20 PIPES, and 3.9 $Ca(Ms)_2$ or 4.8 $Ba(Ms)_2$.

In experiments on the caffeine-induced contraction, the concentration of EGTA was reduced to 0.2 or 0.5 mM.

Measurements of the Phosphorylation of MLC

Skinned muscle strips were used to investigate the phosphorylation of MLC. Tissues (0.02–0.03 mm in thickness, 0.45 mm in width, and 25 mm in length) were prepared using the same procedures as for the tension recordings. Strips were suspended in relaxing solution containing 3 μM A23187 for 10 min to deplete the stored Ca^{2+}; then tissues were skinned in relaxing solution containing 25 μg/ml saponin for 20 min and washed again with relaxing solution. The skinned muscle strips were then suspended in solution containing 0.3 μM Ca^{2+}, 10 μM Ca^{2+}, or 0.1 mM Ba^{2+} buffered with 4 mM EGTA for 3 min and rapidly frozen in liquid nitrogen. The frozen strips were placed in acetone/dry ice and allowed to reach room temperature in acetone. The strips were then homogenized in lysis buffer solution with the following composition: 1% sodium dodecyl sulfate (SDS), 10% glycerol, and 20 mM dithiothreitol (DTT), adjusted to pH 7 with Tris. The volume of lysis buffer solution was 0.1 ml/mg dry tissue weight. Two-dimensional gel electrophoresis involving isoelectric focusing (IEF) in the first dimension and SDS electrophoresis in the second dimension, as developed by O'Farrell (1975), was used for the resolution of MLC phosphorylation. IEF gels with 4% polyacrylamide (2.5 mm in diameter and 110 mm in length) contained 8.5 M urea, 2% Nonidet P-40, and 2% Pharmacia carrier ampholytes (1.6% for pH 4–6.5 and 0.4% for pH 3.5–10). The homogenates (50 μl) were applied and focused at a constant voltage of 100 V for 1 h, 200 V for 2 h, 400 V for 12 h, and 800 V for 1 h. After focusing, the gels were loaded onto the SDS electrophoresis unit. The SDS electrophoresis gels (140 mm in width and 2 mm in thickness) were composed of stacking gels (50 mm in height with 4% polyacrylamide in 0.1% SDS and 0.125 M Tris-HCl at pH 6.8) and separating gels (100 mm in height with 13% polyacrylamide in 0.1% SDS and 0.375 M Tris-HCl at pH 8.8). The gels were run at a constant current density of 20 mA in the stacking gels and 40 mA in the separating gels. The gels were stained overnight with 0.03% Coomassie Brilliant Blue R-250, 50% methanol, and 12% trichloroacetic acid, and then destained with 10% methanol, 7% acetic acid, and 0.85% phosphoric acid. The distribution of the stained protein at 20 kD MLC (LC_{20}) exhibited the first, second, third, and fourth spots from the higher to lower pI values. The intensities of the four spots were measured with a chromatography densitometer equipped with an automatic integrator (CS-910, Shimadzu, Kyoto, Japan). The first area at around pI 5.5 and the second area at around pI 5.45 were measured to obtain the relative value of the MLC phosphorylation, which was expressed as a percent of the second spot area against the sum of the first and second spot areas, as described by Driska et al. (1981).

Drugs

Reagents were as follows: NE-HCl, TFP-2HCl, TTX, and oligomycin (Sigma Chemical Co., St. Louis, MO), guanethidine (Tokyo Kasei Co., Tokyo), caffeine (Wako Pharmaceu-

tical Co., Tokyo), EGTA and PIPES (Dozin Laboratories, Kumamoto, Japan), nisoldipine (Bayer Pharmaceutical Co. Ltd., Basel), $BaCO_3$ and $BaCl_2$ (Nakarai Chemicals Ltd., Kyoto), Ms (Tokyo Kasei Co., Tokyo), ATP (Kojin Co., Ltd., Tokyo), $ATP_\gamma S$ (Boehringer Mannheim, Yamanouchi Co., Tokyo), saponin (ICN Pharmaceuticals Inc., Cleveland, OH), and A23187 (free acid; Calbiochem-Behring Corp., La Jolla, CA).

Statistics

The measured values were expressed as means ± SD (standard deviation) and the number of observations (n). The statistical significance was assessed using Student's t test for unpaired values. P values of <0.05 were considered significant.

RESULTS

Effects of Ba^{2+} on the Mechanical Response in Ca^{2+}-free Solution Containing High K^+

To investigate the effects of 2.6 mM Ba^{2+} on the mechanical response evoked in muscle strips depolarized by 128 mM K^+, a control response was evoked by 128 mM K^+ with 2.6 mM Ca^{2+}, and then the strip was superfused with Ca^{2+}-free (1 mM EGTA) solution containing 128 mM K^+ (3 μM guanethidine and 0.1 μM TTX were added) for 10 min; subsequently, 2.6 mM $BaCl_2$ was repetitively applied for 3 min at 7-min intervals (Fig. 1 A). The amplitude of the Ba^{2+}-induced contraction was progressively decreased with each application of Ba^{2+} until the third trial and then no further reduction occurred (the third contraction was 0.53 ± 0.5 times that induced by 2.6 mM Ca^{2+}, n = 5; Fig. 1 A). The Ba^{2+} contraction evoked by the first trial had a phasic response, with a rapid rate of rise, followed by a tonic contraction, and the shape and amplitude were almost the same as those evoked by 2.6 mM Ca^{2+} (amplitude 0.98 ± 0.3, n = 5; Fig. 1 A). After the third trial, the shape of the contraction was monophasic and had a slow rate of rise. The Ba^{2+}-induced contraction was not modified by the application of 1 μM prazosin or 0.3 μM propranolol (not shown).

Caffeine, in concentrations >1 mM, produced contractions in the presence or absence of Ca^{2+} owing to release of Ca^{2+} from the store sites (Endo, 1977), and 10 mM caffeine had almost no effect on the contractile proteins as estimated from pCa-tension curves in skinned muscle strips (Itoh et al., 1983). When 10 mM caffeine was applied twice, for 2 min each time, at 3-min intervals in Ca^{2+}-free solution containing 128 mM K^+, there was a minute contraction on the second application caused by depletion of the stored Ca^{2+} (Fig. 1 B). When 2.6 mM Ba^{2+} was applied repeatedly for 3 min, each resulting contraction had a constant amplitude in all trials (Fig. 1 B). Fig. 1 C shows the effect of Ba^{2+} on the contraction using the combined experimental procedures as described in Fig. 1, A and B. In Ca^{2+}-free (1 mM EGTA) solution containing 128 mM K^+, 2.6 mM Ca^{2+} was applied for 3 min and then a contraction was evoked in Ca^{2+}-free solution by caffeine, which depleted the Ca^{2+} stored in the cells. 2.6 mM Ca^{2+}, subsequently applied, produced the same amplitude of contraction as observed before caffeine. When 2.6 mM Ba^{2+} was applied, the amplitude and shape of the contraction were the same as those observed in Fig. 1 A. 10 mM caffeine, subsequently applied, produced only a minute contraction and Ba^{2+} then produced a small tonic contraction, as shown in Fig. 1 A. These results indicate that

the generation of the phasic response by Ba^{2+} requires loading of Ca^{2+} into the store site, and Ba^{2+} seems not to be accumulated into the store site.

Effects of Nisoldipine on Ba^{2+}- or Ca^{2+}-induced Contractions

Figs. 2 and 3 show the effect of nisoldipine, a Ca^{2+} channel blocking agent, on the concentration-response relationship for Ca^{2+} and Ba^{2+} in Ca^{2+}-free solution
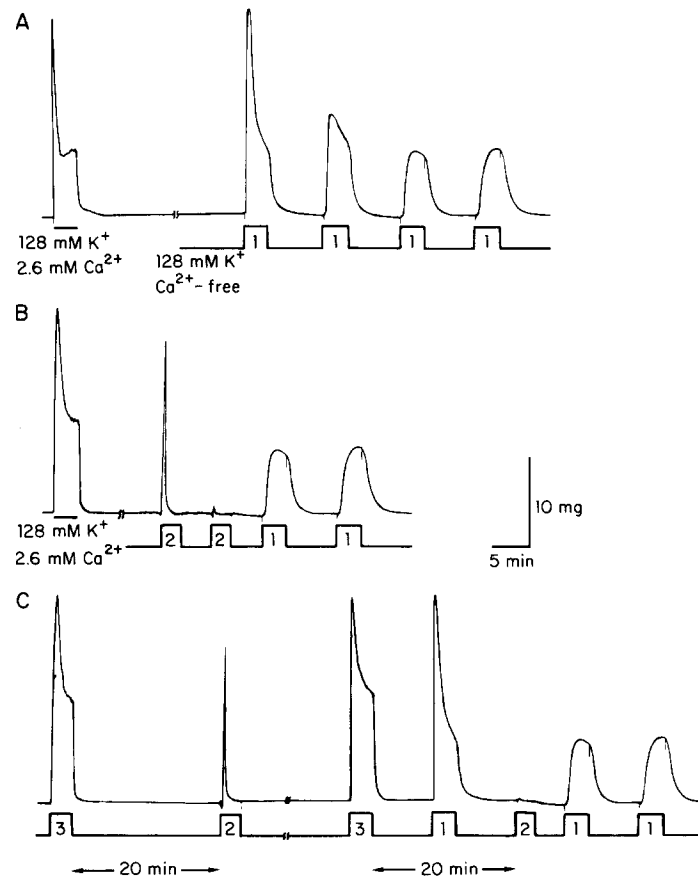

FIGURE 1. Effects of Ba^{2+}, Ca^{2+}, or caffeine on the muscle strip depolarized by 128 mM K^+ in Ca^{2+}-free solution containing 1 mM EGTA. (A) As the control, contraction was evoked by a solution containing 2.6 mM Ca^{2+} and 128 mM K^+. Ca^{2+}-free (1 mM EGTA) solution containing 128 mM K^+ was then applied 10 min before repeated applications of 2.6 mM Ba^{2+} (solution 1; 3 min with 7-min intervals). (B) After recording the control contraction (2.6 mM Ca^{2+} with 128 mM K^+), 10 mM caffeine (solution 2) was applied twice; then 2.6 mM Ba^{2+}, 128 mM K^+ solution was applied twice. (C) After depletion of Ca^{2+} stored in cells by application of 10 mM caffeine in Ca^{2+}-free (1 mM EGTA) solution containing 128 mM K^+ (not shown), 2.6 mM Ca^{2+} (solution 3) was applied for 3 min; 20 min later, 10 mM caffeine was applied. After 30 min, 2.6 mM Ca^{2+} was again applied for 3 min, and then 2.6 mM Ba^{2+} or 10 mM caffeine was applied.

(1 mM EGTA) containing 128 mM K^+. The muscle strips were exposed to Ca^{2+}-free K^+ solution for 60 min after repetitive application of 10 mM caffeine. Various concentrations of Ca^{2+} and Ba^{2+} (0.16–5.2 mM) were then applied for 3 min at 7-min intervals (Figs. 2A and 3A). The amplitudes of the Ca^{2+}- and
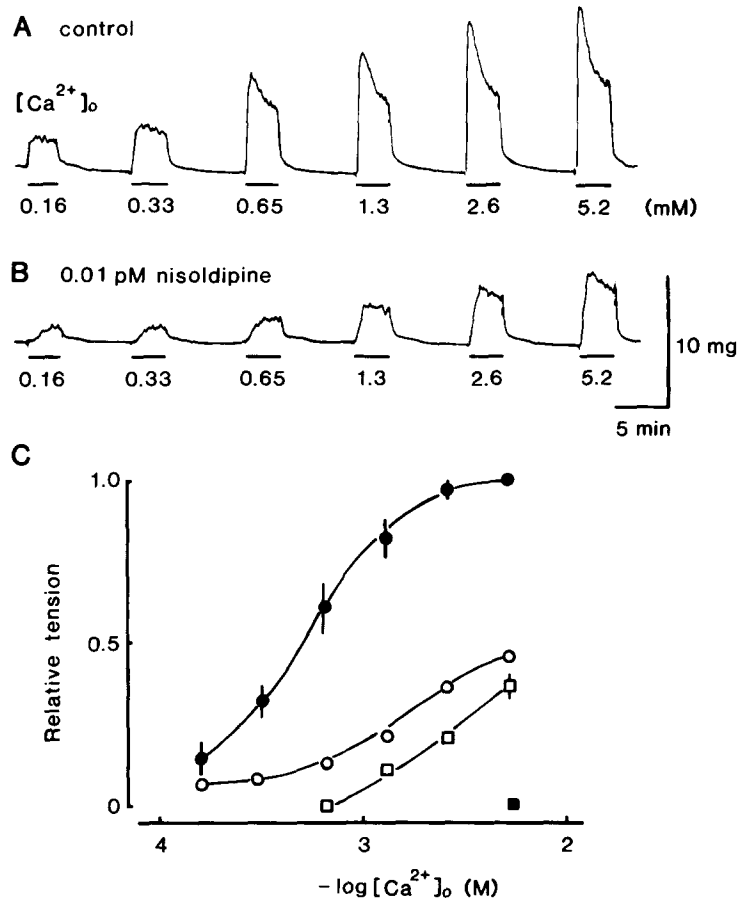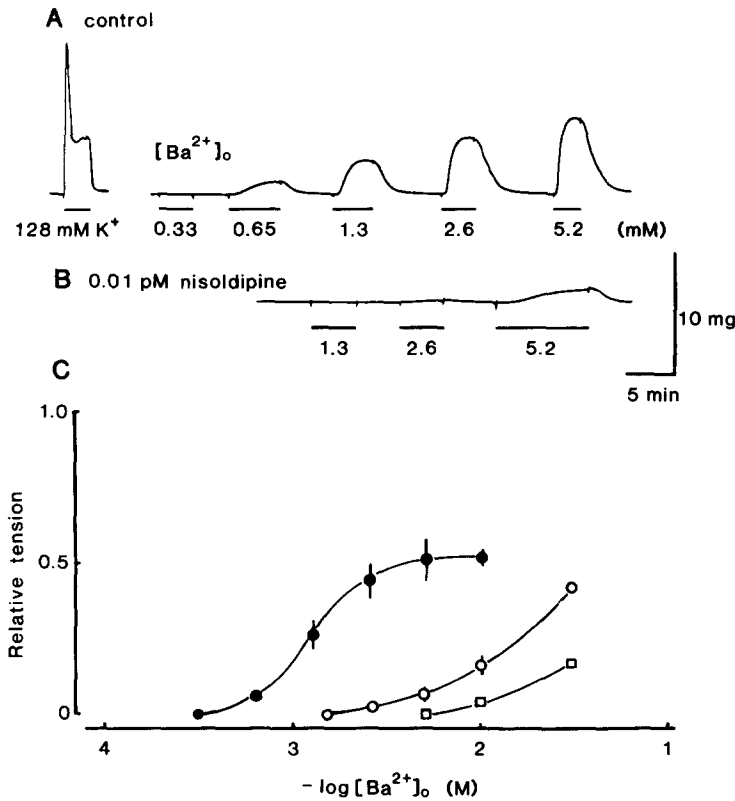

FIGURE 2.   Effects of nisoldipine on the Ca^{2+}-induced contraction in the muscle strips depolarized by 128 mM K^+. After the muscle strips were depolarized by 128 mM K^+ in Ca^{2+}-free solution, various concentrations of Ca^{2+} were applied. (A) Control; (B) in the presence of 0.01 pM nisoldipine; (C) dose-response relationships observed before and after application of various concentrations of nisoldipine (control [●], 0.01 pM [○], 0.1 nM [□], 10 nM [■]). The contraction evoked by 5.2 mM Ca^{2+} was normalized, as the control, to 1.0 ($n = 3$).

Ba^{2+}-induced contractions increased in a dose-dependent manner. When nisoldipine (0.01 pM) was applied, both the phasic and tonic contractions evoked by Ca^{2+} were inhibited, but the phasic contraction was inhibited to a greater extent (Fig. 2B). In the case of Ba^{2+}, nisoldipine abolished the monophasic contraction (Fig. 3B). To observe the effects of nisoldipine (0.01 pM to 10 nM) on the dose-

response relationship for Ca^{2+} or Ba^{2+}, the maximum amplitude of the contraction evoked by 5.2 mM Ca^{2+} was normalized as the control (1.0 in Fig. 2C and 3C). The concentration of Ba^{2+} was increased up to 30 mM when nisoldipine was applied. Nisoldipine inhibited the contraction evoked by Ba^{2+} more than that evoked by Ca^{2+}.

Fig. 4 shows the effects of 10 nM nisoldipine on the contraction evoked by 128 mM K^+ or 10 μM NE in the solution containing 2.6 mM Ca^{2+} or Ba^{2+}. In

FIGURE 3.   Effects of nisolidipine on the Ba^{2+}-induced contraction in the muscle strips depolarized by 128 mM K^+. After the muscle strips were depolarized by 128 mM K^+ in Ca^{2+}-free solution, various concentrations of Ba^{2+} were applied. (A) Control; (B) in the presence of 0.01 pM nisolidipine; (C) dose-response relationships observed before and after application of various concentrations of nisoldipine (control [●], 0.01 pM [○], 0.1 nM [□]). The contraction evoked by 5.2 mM Ca^{2+} was normalized, as the control, to 1.0 (n = 3).

Krebs solution (2.6 mM Ca^{2+} and 5.9 mM K^+), the contraction evoked by 128 mM K^+ was composed of phasic and tonic responses and that evoked by NE was composed of phasic and subsequent repetitive oscillatory responses on the tonic response (Itoh et al., 1983; Fig. 4A). Nisoldipine (10 nM) markedly inhibited the tonic response but only slightly inhibited the phasic response evoked by 128 mM K^+, whereas both responses evoked by NE were slightly inhibited and the

oscillatory contraction ceased (Fig. 4*A*). When 2.6 mM Ba^{2+} was applied in Ca^{2+}-free (1 mM EGTA) solution containing 5.9 mM K^+ after depletion of the Ca^{2+} stored in cells by repetitive applications of 10 mM caffeine, the resting tension was slightly raised (Fig. 4*B*). When 128 mM K^+ was applied in the presence of 2.6 mM Ba^{2+}, monophasic contractions with slow rates of rise and fall were recorded. Upon application of 10 μM NE, there was an even greater reduction in the amplitude and rates of rise and fall in comparison with those observed in the presence of 2.6 mM Ca^{2+} (Fig. 4*B*). Nisoldipine (10 nM) abolished the contraction evoked by 128 mM K^+ or NE in the presence of 2.6 mM Ba^{2+} (Fig. 4*B*). These observations support the previous suggestion that Ba^{2+} may not
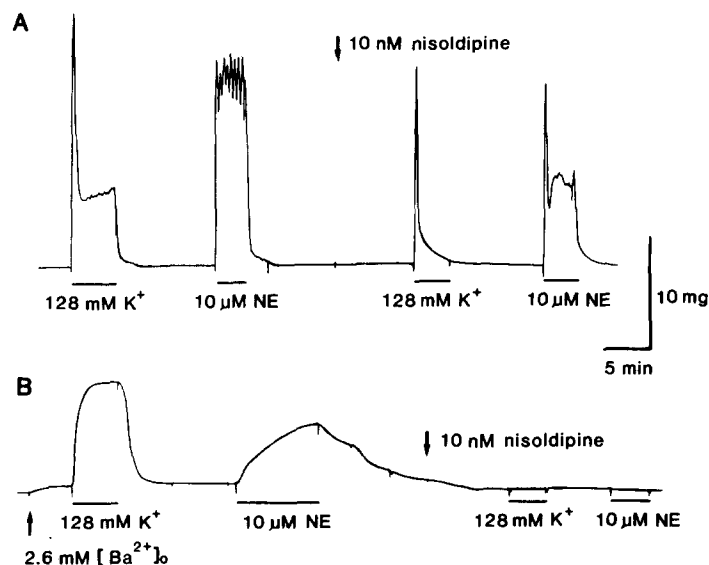

FIGURE 4. Effects of nisoldipine on the K^+-induced (128 mM) and norepinephrine-induced (10 μM) contractions. (*A*) Responses were evoked in the presence of 2.6 mM Ca^{2+}. (*B*) Responses were evoked in solution containing 2.6 mM Ba^{2+}.

accumulate in the store site. As a consequence, the phasic (K^+ or NE) and oscillatory (NE) contractions do not occur. Furthermore, Ba^{2+} may not permeate the receptor-operated Ca^{2+} channel, which was activated by the α_1-adrenoceptor, as estimated from the action of the Ca antagonist nisoldipine.

Effects of Ba^{2+} on the Contractile Proteins as Estimated from the Contraction Evoked in Skinned Muscles

Figs. 5 and 6 show the pCa- and pBa-tension relationships observed in skinned muscle strips. The minimum concentrations of Ca^{2+} and Ba^{2+} required to produce a contraction were 0.1 and 10 μM, respectively. Cumulatively increased concentrations of Ca^{2+} or Ba^{2+} enhanced the amplitude of the contraction up to 3 μM for Ca^{2+} or 3 mM for Ba^{2+} (Figs. 5*B* and 6*B*). The amplitude of the contraction evoked by 3 mM Ba^{2+} was 0.92 ± 0.3 times (n = 5) the contraction evoked by 10 μM Ca^{2+} (Fig. 5*C* vs. 6*C*). The half-maximal amplitudes of contraction were

obtained with 0.34 ± 0.03 μM Ca^{2+} and 90 ± 5 μM Ba^{2+}, and the Hill coefficients were 1.8 ± 0.1 and 1.0 ± 0.1, as estimated from the pCa- and pBa-tension relationships, respectively ($n = 5$). The maximum amplitude of the Ca^{2+}- or Ba^{2+}-induced contraction was consistently larger than that evoked by 128 mM K^+ in intact muscle strips (panels A and B of Figs. 5 and 6). Since contractions induced by caffeine (25 mM) or A23187 (1 μM) are abolished in relaxing solution containing 4 mM EGTA (Itoh et al., 1984), Ca^{2+} released from store sites probably does not contribute to the pCa- or pBa-tension relationships.
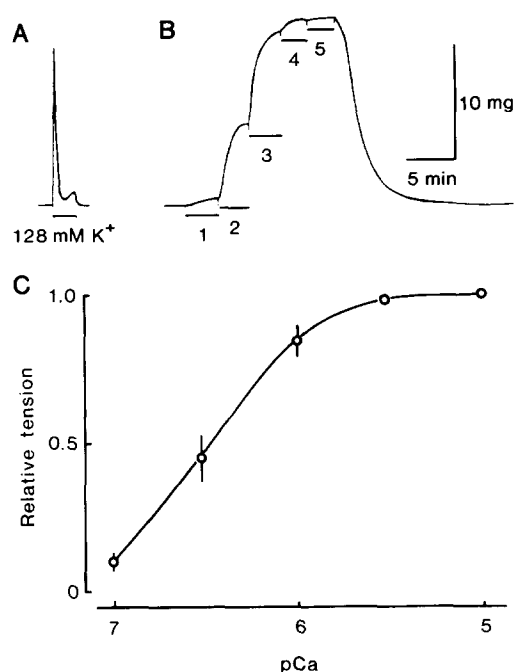

FIGURE 5. Ca^{2+}-induced contractions in skinned muscle strips prepared by 25 μg/ml saponin. (A) Before application of saponin, 128 mM K^+ was applied to evoke the contraction as the control. (B) After skinning the tissue (see Methods), various concentrations of Ca^{2+} (1, 0.1, 2, 0.3, 3, 1.0, 4, 3.0, 5, and 10 μM) were cumulatively applied. (C) The pCa-tension relationship. The line was drawn as described in the Methods. From these relations, the Hill coefficient was obtained (see the text). The amplitude of the contraction evoked by 10 μM Ca^{2+} was normalized to 1.0 ($n = 5$).

Fig. 7 shows interactions of Ca^{2+} and Ba^{2+} on the contraction evoked in skinned muscle strips. The amplitude of the contraction evoked by 0.3 μM Ca^{2+} was enhanced by the addition of Ba^{2+} (Fig. 7B). In Fig. 7C, the effects of two different concentrations of Ca^{2+} on the Ba^{2+}-induced contraction (10 μM to 0.3 mM) are shown. Each divalent cation additively enhanced the amplitude of the contraction produced by the other up to the amplitude of contraction evoked by 1 μM Ca^{2+}.

It is established that Ca^{2+} binds to calmodulin and phosphorylates MLC as a result of activation of MLC kinase (MLCK), and initiates contraction in vascular smooth muscle (Adelstein and Eisenberg, 1980). To investigate the role of calmodulin in the Ba^{2+}-induced contraction, the effects of exogenously applied calmodulin and TFP, which enhance and depress, respectively, the activity of MLCK, were observed. Fig. 8 shows the effects of TFP on the contraction evoked by 0.3 and 10 μM Ca^{2+} or 1 mM Ba^{2+} in skinned muscle strips. When three different concentrations of TFP (30 μM, 0.1 mM, and 0.3 mM) were cumulatively
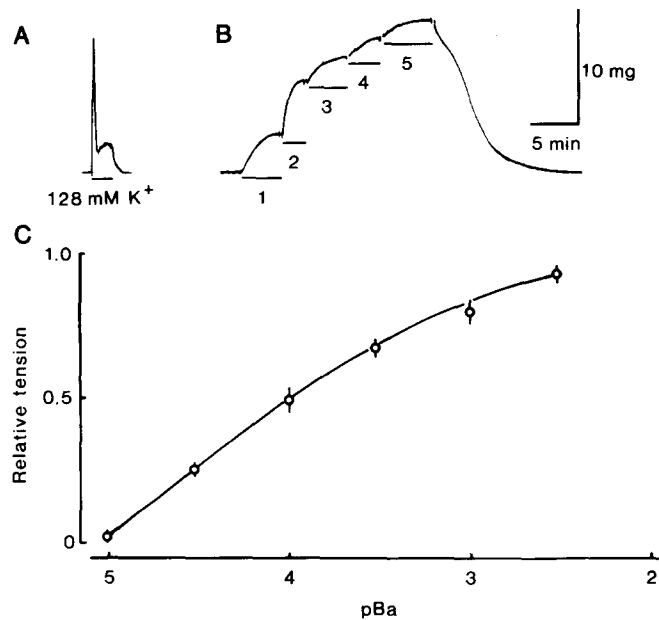

FIGURE 6. Ba^{2+}-induced contractions in skinned muscle strips prepared by 25 μg/ml saponin. (A) Before application of saponin, 128 mM K^+ was applied to evoke the contraction as the control. (B) After skinning the tissue, various concentrations of Ba^{2+} (solutions: 1: 0.03; 2: 0.3; 3: 1.0; 4: 3.0 mM) and 10 μM Ca^{2+} (solution 5) were cumulatively applied. (C) The pBa-tension relationship. The line was drawn as described in the Methods. From these relations, the Hill coefficient was obtained. The amplitude of the contraction evoked by 10 μM Ca^{2+} was normalized to 1.0 (n = 5).

applied after the Ba^{2+}-induced contraction (1 mM) reached a steady level, the muscle strip relaxed in a dose-dependent manner (Fig. 8B). Fig. 8C summarizes the effects of TFP on the Ba^{2+}- and Ca^{2+}-induced contractions. The amplitude of the contraction evoked by each stimulant (0.3 μM Ca^{2+} and 10 μM Ca^{2+} or 1 mM Ba^{2+}) was normalized to 1.0. The ID_{50} values of TFP, as estimated from the inhibition of the contraction evoked by 0.3 μM Ca^{2+}, 10 μM Ca^{2+}, and 1 mM Ba^{2+}, were 16 ± 3, 40 ± 12, and 25 ± 10 μM, respectively (n = 4). When the ID_{50} values were compared for contractions evoked by 10 μM Ca^{2+} and 1 mM Ba^{2+}, there was no significant difference (P > 0.05).

Fig. 9 shows the effects of calmodulin on the Ca^{2+}- and Ba^{2+}-induced contractions in skinned muscle strips. When 0.3 μM calmodulin was added during the generation of the contraction evoked by 0.1 mM Ba^{2+}, the contraction was slowly enhanced. On increasing the concentration of calmodulin, the tension was increased further (Fig. 9A). Fig. 9B summarizes the effects of calmodulin on the contraction evoked by 0.3 μM Ca^{2+} and 0.1 mM Ba^{2+}. To compare the amplitudes
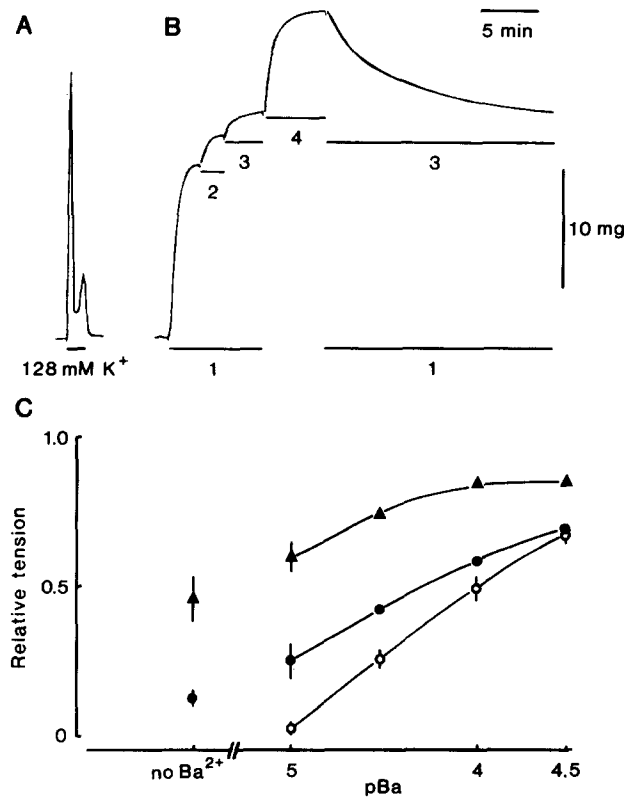

FIGURE 7. Interactions of Ca^{2+} or Ba^{2+} on the contraction evoked in skinned muscle strips. (A) The contraction evoked by 128 mM K^+ with 2.6 mM Ca^{2+} before the application of saponin. (B) After skinning the muscle strip with saponin, Ba^{2+} was cumulatively applied after application of 0.3 μM Ca^{2+} for 2 min (solutions: 1: 0.3 μM Ca^{2+}; 2: 10 μM Ba^{2+}; 3: 1 mM Ba^{2+}; 4: 10 μM Ca^{2+}). (C) Effects of two different concentrations of Ca^{2+} on the Ba^{2+}-induced contraction (with no Ca^{2+} [O], 0.1 μM Ca^{2+} [●], 0.3 μM Ca^{2+} [▲]). Concentrations of Ba^{2+} varied from 10 μM to 0.3 mM and were added cumulatively. The amplitude of the contraction induced by 10 μM Ca^{2+} was normalized to 1.0 ($n = 4$).

of the contractions evoked by Ba^{2+} and Ca^{2+}, the 10 μM Ca^{2+}-induced contraction was normalized to 1.0. Calmodulin (0.1–3 μM) had much the same action on both contractions. As shown in Fig. 10, calmodulin (3 μM) in relaxing solution also enhanced the contraction evoked by cumulatively applied Ca^{2+} or Ba^{2+} to the same extent. However, 3 μM calmodulin did not modify the maximum amplitude of the contraction evoked by 10 μM Ca^{2+}.

When $MgATP_\gamma S$ with Ca^{2+} is applied to skinned muscle strips in ATP-free solution, irreversible MLC phosphorylation occurs, and an additional application of MgATP produces contraction in Ca^{2+}-free solution (Ca^{2+}-independent contraction: Cassidy et al., 1979; Walsh et al., 1983). To compare the actions of Ba^{2+} and Ca^{2+} on the contractile machinery, we observed the effects of Ba^{2+} on the Ca^{2+}-independent contraction using the following procedures: after skinning the muscle strips, a rigor solution (ATP-, Ca^{2+}-, and Ba^{2+}-free and containing 4
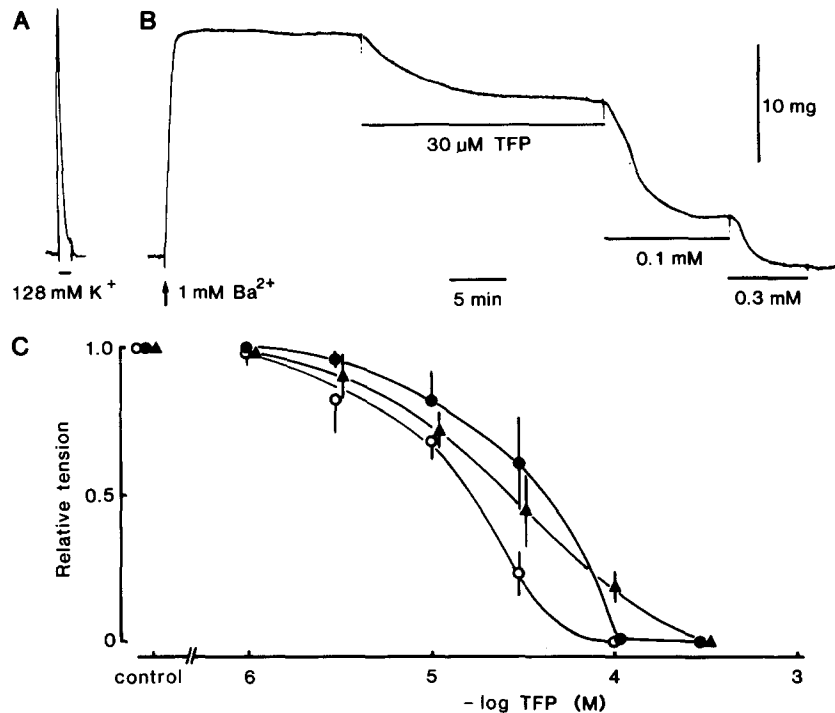

FIGURE 8. Effects of TFP on Ca^{2+}- and Ba^{2+}-induced contractions in skinned muscle strips. (A) K^+-induced contraction. (B) Effects of TFP on the contraction evoked by 1 mM Ba^{2+}. After the Ba^{2+}-induced contraction had reached a steady amplitude, 30 μM, 0.1 mM, and 0.3 mM TFP were cumulatively applied. (C) Dose-response relationships of TFP observed on the Ca^{2+}- or Ba^{2+}-induced contraction. The contractions evoked by 0.3 μM Ca^{2+} (○), 10 μM Ca^{2+} (●), and 1 mM Ba^{2+} (▲) without TFP were normalized to 1.0 ($n = 4$). The concentrations of TFP varied from 1 μM to 0.3 mM.

mM EGTA) was applied for 3 min to eliminate ATP from the muscle cell, and the solution containing 10 μM Ca^{2+} or 1 mM Ba^{2+} with 2 mM $ATP_\gamma S$ was applied for 5 min. The skinned muscle strip was rinsed again with the rigor solution for 2 min and the solution containing 4 mM MgATP (relaxing solution; see Methods) was applied to provoke the Ca^{2+}-independent contraction. Finally, 10 μM Ca^{2+} was applied. As shown in Fig. 11, during the addition of 10 μM Ca^{2+} or 1 mM Ba^{2+} with 2 mM $ATP_\gamma S$, and upon application of the relaxing solution, a contraction was evoked. The amplitude of the Ca^{2+}- (Ba^{2+}-) independent con-

traction evoked by the relaxing solution was almost the same as that evoked by 10 μM Ca^{2+} (Fig. 11, A and B). These results indicate that Ba^{2+} may bind to calmodulin, thus producing a contraction through thiophosphorylation of the MLC as Ca^{2+} does.

To investigate the action of Ba^{2+} on MLC phosphorylation, we measured the MLC phosphorylation by two-dimensional gel electrophoresis (see Methods). Fig. 12 shows the gel profiles and Table I summarizes the effect of 0.3 μM, 10 μM Ca^{2+}, and 0.1 mM Ba^{2+} on the MLC phosphorylation. In relaxing solution (4 mM EGTA), the phosphorylation of MLC was small (5.2%) and the amounts of
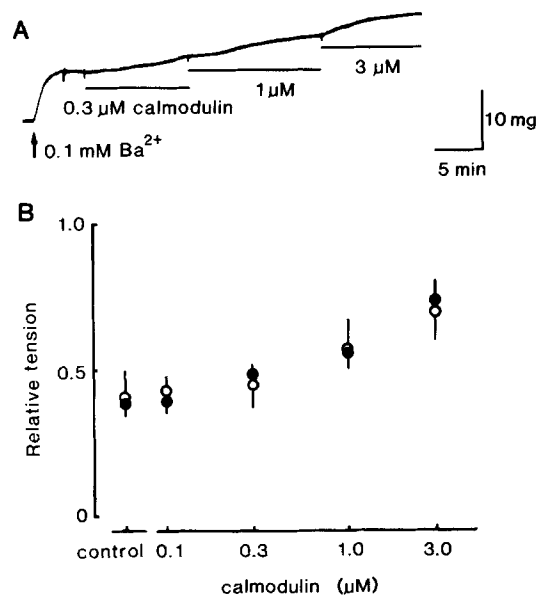

FIGURE 9.   Effects of exogenously applied calmodulin on the Ca^{2+}- and Ba^{2+}-induced contractions in skinned muscle strips. (A) Effects of cumulatively applied calmodulin (0.3–3 μM) on the contraction evoked by 0.1 mM Ba^{2+}. (B) Dose-response relationship of calmodulin (0.1–3 μM) observed on the 0.3 μM Ca^{2+}-induced (O) or 0.1 mM Ba^{2+}-induced (●) contraction. The amplitude of the contraction evoked by 10 μM Ca^{2+} was normalized to 1.0 for both contractions ($n = $ 4).

phosphorylation increased with the application of 0.3 μM Ca^{2+} to 28%, with 10 μM Ca^{2+} to 62%, and with 0.1 mM Ba^{2+} to 32%. The results indicated that 0.1 mM Ba^{2+} phosphorylated the MLC to the same extent as 0.3 μM Ca^{2+}. Furthermore, as described previously in skinned muscle strips, the amplitudes of the contractions evoked by 0.3 μM Ca^{2+} and 0.1 mM Ba^{2+} were very similar.

Effects of Ba^{2+} on the Release of Ca^{2+} from Store Sites as Estimated from the Caffeine-induced Contraction in Skinned Muscle Strips

The results obtained in intact muscle strips suggested that Ba^{2+} may act on the store site. To clarify further the action of Ba^{2+} on the store site, the effects of Ba^{2+} on the caffeine-induced contraction in skinned muscle strips were investi-
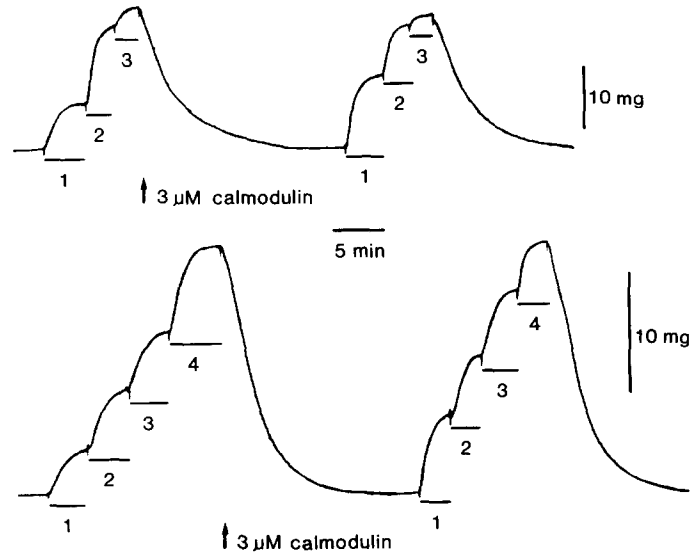
FIGURE 10.   The cumulatively evoked Ca^{2+}- or Ba^{2+}-induced contraction before and after application of 3 μM calmodulin. After the control responses were observed, 3 μM calmodulin was added to the relaxing solution, and after complete relaxation, Ca^{2+} or Ba^{2+} was cumulatively applied (upper trace: 1: 0.3 μM Ca^{2+}; 2: 1 μM Ca^{2+}; 3: 10 μM Ca^{2+}; lower trace: 1: 0.03 mM Ba^{2+}; 2: 0.3 mM Ba^{2+}; 3: 1 mM Ba^{2+}; 4: 10 μM Ca^{2+}).

gated using the following procedures: 0.3 μM Ca^{2+} or 0.1 mM Ba^{2+}, buffered with 4 mM EGTA, was applied for 1 min. Subsequently, relaxing solution containing 4 mM EGTA was applied for 10 s to rapidly remove Ca^{2+} (or Ba^{2+}) and again the strip was rinsed with relaxing solution containing 0.2 mM EGTA for 2.5 min. Finally, 25 mM caffeine was applied to estimate the amount of stored Ca^{2+} from the amplitude of the caffeine-induced contraction. To prevent the contribution of Ca^{2+} accumulated in the mitochondria, 5 mM NaN_3 or 5 μg/ml oligomycin was applied throughout the experiments.
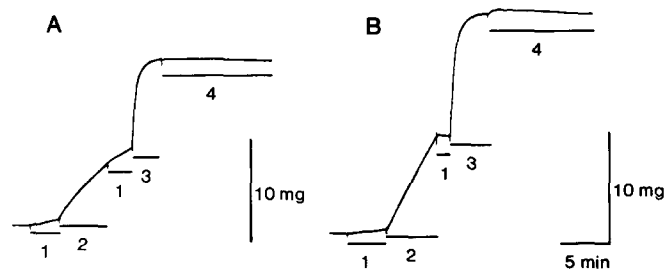

FIGURE 11.   Effects of Ca^{2+} or Ba^{2+} on the Ca^{2+}-independent contraction in the presence of ATP$_\gamma$S in skinned muscle strips. Experimental procedures are indicated in the figure and described in the text. Solutions: 1: rigor solution; 2: 10 μM Ca^{2+} with 2 mM ATP$_\gamma$S (A) or 1 mM Ba^{2+} with 2 mM ATP$_\gamma$S (B); 3: relaxing solution; 4: 10 μM Ca^{2+}.
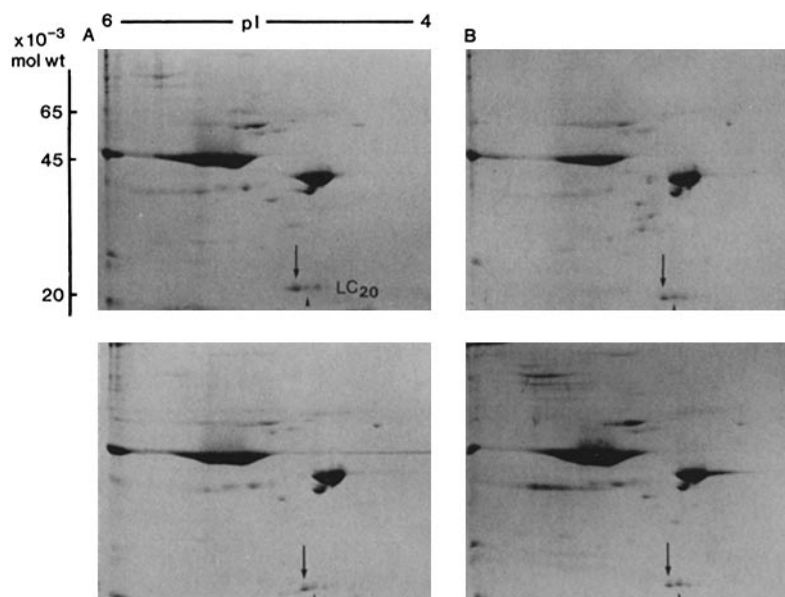
FIGURE 12.   Two-dimensional gel electrophoretic analysis of MLC phosphorylation in saponin-treated, chemically skinned muscle strips of the rabbit mesenteric artery. The skinned muscle strips were immersed in the solution containing 4 mM EGTA and no Ca^{2+} (A), 0.3 μM Ca^{2+} (B), 0.1 mM Ba^{2+} (C), or 10 μM Ca^{2+} (D) for 3 min. The gel staining profiles of the arterial proteins including the 20,000-dalton MLC (LC_{20}) are shown. The arrows and arrowheads indicate the dephosphorylated and phosphorylated MLC's, respectively (see also Methods).

When 0.3 μM Ca^{2+} was applied to skinned muscle strips, a large caffeine-induced contraction was provoked (Fig. 13A, part a). However, although applied 0.1 mM Ba^{2+} itself produced a contraction, subsequently applied caffeine did not (Fig. 13A, part b). These results indicate either that Ba^{2+} is not accumulated by the caffeine-sensitive store or that Ba^{2+} is accumulated in the SR, but is not released by caffeine. To clarify the above possibilities, 1 μM A23187, a nonselective releaser of divalent cations from the SR (Itoh et al., 1985), was applied after the application of Ca^{2+} or Ba^{2+} to skinned muscle strips. After the applica-

TABLE I

Phosphorylation of MLC in Skinned
Muscle Strips in the Presence of Ca^{2+} or Ba^{2+}

Concentration	Percent phosphorylation
Relaxing solution (4 mM EGTA)	5.2±2.5
0.3 μM Ca^{2+}	28.3±3.2
10 μM Ca^{2+}	62.0±6.8
0.1 mM Ba^{2+}	32.1±5.6

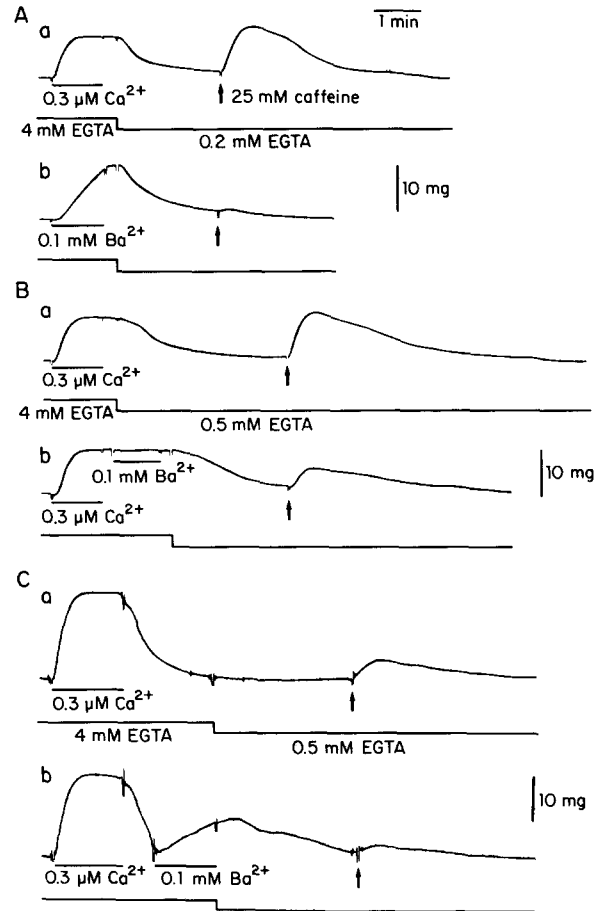The conditions of the assay are described in the Methods. Values are means ± SD (n = 3).

FIGURE 13.   Effects of Ba^{2+} or Ca^{2+} on the caffeine-induced contraction in skinned muscle strips. (*A*) After skinning the tissue, 0.3 μM Ca^{2+} (*a*) or 0.1 mM Ba^{2+} (*b*) was applied for 1 min, and after the tissue was relaxed to the resting level, 25 mM caffeine was applied. The arrows indicate the applications of 25 mM caffeine. (*B*, part *a*) The experimental procedures were almost the same as *A*, part *a*; the control was the same as for *B*, part *b*. (*B*, part *b*) After the application of 0.3 μM Ca^{2+} for 1 min and removal of Ca^{2+} with Ca^{2+}-free solution containing 4 mM EGTA for 10 s, 0.1 mM Ba^{2+} was applied for 1 min; then the tissue was rinsed with the relaxing solution and 25 mM caffeine was applied for the same interval as applied in *B*, part *a* (from the onset of Ca^{2+} application). During the application of Ca^{2+} or Ba^{2+}, 4 mM EGTA was added to the solution and the relaxing solution contained 0.2 mM (*A*) or 0.5 mM (*B*) EGTA. (*C*) After the application of 0.3 μM Ca^{2+} for 1.5 min, the tissue was rinsed with the relaxing solution containing 4 mM EGTA to accelerate the removal of free Ca^{2+}. Subsequently, 0.1 mM Ba^{2+} was applied for 1 min and the tissue was then rinsed with the relaxing solution containing 0.5 mM EGTA. When 25 mM caffeine was applied with the same interval as applied in the control (*C*, part *a*), the much smaller amplitude of the contraction was evoked (*C*, part *b*). Oligomycin (5 μg/ml) was added throughout the experiment.

tion of 0.1 mM Ba^{2+}, A23187 evoked a very small contraction (0.1 times the caffeine-induced contraction after the application of 0.3 μM Ca^{2+}), but this ionophore produced a large contraction after the application of 0.3 μM Ca^{2+} (1.2 times the caffeine-induced contraction). Thus, Ba^{2+} may not be accumulated in the SR. Fig. 13B shows the effects of Ba^{2+} on Ca^{2+} release from the store site (SR) observed in skinned muscle strips. The experimental procedures were essentially the same as those described in Fig. 13A (but to minimize the concentration of contaminating Ca^{2+}, 0.5 mM EGTA was used instead of 0.2 mM EGTA in the relaxing solution). Ba^{2+} (0.1 mM) was applied for 1 min after the application of 0.3 μM Ca^{2+} for 1 min, and 25 mM caffeine was then applied after the application of Ca^{2+}-free solutions (4 mM EGTA-containing solution for 10 s and 0.5 mM EGTA-containing solution for 2.5 min). Since 0.3 μM Ca^{2+} and 0.1 mM Ba^{2+} produced a contraction of about the same amplitude as that in skinned muscle strips, subsequently applied 0.1 mM Ba^{2+} maintained the contraction evoked by 0.3 μM Ca^{2+} at the same level (Figs. 5, 6, and 13B, part b). However, the caffeine-induced contraction was inhibited after the application of Ba^{2+} (0.38 ± 0.1 times the caffeine-induced contraction observed in the absence of Ba^{2+}, n = 3). To determine whether Ba^{2+} releases Ca^{2+} from the store site or activates Ca^{2+}-induced release of Ca^{2+} resulting from the replacement of Ca^{2+} from EGTA by Ba^{2+}, the following experiment was performed: after the application of 0.3 μM Ca^{2+}, the skinned muscle strip was completely relaxed by removal of Ca^{2+} with Ca^{2+}-free solution containing 4 mM or 0.5 mM EGTA, and 0.1 mM Ba^{2+} buffered with 4 mM EGTA was applied for 1 min. Subsequently applied caffeine (25 mM) evoked a contraction smaller than that of the control (Fig. 13C). These observations also suggest that Ba^{2+} is not accumulated into the store site and, furthermore, that Ba^{2+} may release Ca^{2+} accumulated in the store site.

DISCUSSION

There is considerable electrophysiological evidence to support the idea that Ba^{2+} can pass through the voltage-dependent Ca^{2+} channel at plasma membranes, including those of smooth muscle (for example, barnacle muscle, Hagiwara et al., 1974; skeletal muscle, Potreau and Raymond, 1980; cardiac muscle, Reuter, 1983; Lee and Tsien, 1983; smooth muscle, Bülbring and Tomita, 1969; Inomata and Kao, 1985; Benham et al., 1985; for review, see also Hagiwara and Byerly, 1981). Somlyo et al. (1974) showed that incubation of vascular smooth muscle with Ba^{2+} resulted in the accumulation of mitochondrial granules, presumably owing to transmembrane Ba^{2+} fluxes. Our results showed that the Ba^{2+}-induced contraction was more enhanced in Ca^{2+}-free solution containing 128 mM K^+ than in solution with 5.9 mM K^+, and that both responses were abolished by the Ca^{2+} channel antagonist nisoldipine (Fig. 4B). These findings indicate that Ba^{2+} permeates the voltage-dependent Ca^{2+} channel in this smooth muscle tissue.

In rabbit mesenteric artery, exogenously applied NE at high concentrations (>1.0 μM) produced contraction owing to activation of three processes, i.e., Ca^{2+} release from the store site, voltage-dependent Ca^{2+} influx, and receptor-operated Ca^{2+} influx (Kanmura et al., 1983, 1984; Itoh et al., 1984; Hashimoto et al., 1986). The voltage-dependent and receptor-operated Ca^{2+} influxes can be dis-

tinguished from each other by the application of dihydropyridine derivatives (nifedipine or nisoldipine; Kanmura et al., 1983; Itoh et al., 1984). Since the NE-induced contraction in Ba^{2+}-containing solution, but not in Ca^{2+}-containing solution, was completely inhibited by nisoldipine, Ba^{2+} may not pass through the receptor-operated Ca^{2+} channel.

There are several mechanisms postulated for the Ba^{2+}-induced contraction in intact muscle tissues. Ba^{2+} may increase the release of NE from sympathetic nerve endings, thus enhancing the muscle activity (Hansen et al., 1984), may activate Ca^{2+} release from the store site (Somlyo et al., 1974), and may directly activate the contractile machinery of the arterial smooth muscle (Hansen et al., 1984). However, the first possibility can be ruled out because the Ba^{2+}-induced contraction is evoked in the presence of 3 μM guanethidine and 0.1 μM TTX.

Northover (1968) and Somlyo et al. (1974) reported that the Ba^{2+}-induced contraction was due to the release of Ca^{2+} from store sites, presumably the SR. In the present experiments, the Ba^{2+}-induced contraction in 128 mM K^+, Ca^{2+}-free solution gradually decreased with successive applications of Ba^{2+}. After the third application, when the cellular storage of Ca^{2+} was reduced to near the minimum, the amplitude of the contractions was constant. If Ca^{2+} was applied once instead of Ba^{2+}, a large phasic contraction with a fast rate of rise was evoked by the following application of Ba^{2+}. Therefore, Ba^{2+} may release Ca^{2+} stored in smooth muscle cells to produce the phasic contraction. Furthermore, it was also confirmed that Ba^{2+} releases Ca^{2+} from store sites in skinned muscle strips (Fig. 13B, part b). The tonic contraction with a slow rate of rise induced by Ba^{2+} is presumably due to direct action of Ba^{2+} on the contractile proteins without involvement of the store site that is responsible for the accumulation of Ca^{2+}. These possibilities were confirmed by the observations made on skinned muscle strips, since caffeine did not produce a contraction after the application of Ba^{2+} (Fig. 13A, part b).

Somlyo and Somlyo (1971) observed that Ba^{2+} was not accumulated into the SR in smooth muscle cells of rabbit portal vein. Our results indicate that Ba^{2+} releases Ca^{2+} from the SR, but is not taken up by the SR. Thus, the behavior of Ba^{2+} differed from that of Ca^{2+} and Sr^{2+}. The agonist- (for example, NE or acetylcholine) induced release of Ca^{2+} from the store site in vascular smooth muscles was postulated to be induced by Ca^{2+} or caused by inositol 1,4,5-trisphosphate (Itoh et al., 1981, 1985; Suematsu et al., 1984; Hashimoto et al., 1986). The contraction evoked by NE that was due to release of Ca^{2+} from the store site in cells and to the receptor-operated Ca^{2+} influx was not affected by nisoldipine (Kanmura et al., 1983; Itoh et al., 1984). Since NE produced only a tonic contraction, which was abolished by nisoldipine, with a slow rate of rise in the presence of Ba^{2+}, this NE-induced contraction may not be generated by activation of the receptor-operated Ca^{2+} channel or by release of Ba^{2+} from the store site activated by inositol 1,4,5-trisphosphate, but by the voltage-dependent Ba^{2+} influx, which is selectively sensitive to nisoldipine.

Hansen et al. (1984) reported that the Ba^{2+}-induced contraction in Ca^{2+}-depleted aortic strips was due to direct activation of the contractile machinery by Ba^{2+} itself. Ba^{2+} produced contraction of skinned muscle strips and superpre-

cipitated myosin B with a low affinity for the contractile machinery in comparison with Ca^{2+} (Ebashi and Endo, 1968; Kreye et al., 1986). There is much evidence that actomyosin ATPase activity of isolated contractile proteins of smooth muscle cells or contraction in skinned muscles activated by Ca^{2+} is largely dependent on the level of the MLC phosphorylation, as a result of production of the Ca^{2+}-calmodulin complex, which activates MLCK (see reviews by Adelstein and Eisenberg, 1980; Hartshorne and Mrwa, 1982). Our observations showed that calmodulin enhanced and TFP inhibited the Ba^{2+}-induced contraction. Furthermore, the contraction was provoked by the application of MgATP in the absence of Ba^{2+} and Ca^{2+} after the application of $ATP_\gamma S$ with Ba^{2+}, similar to Ca^{2+}-independent contraction (Cassidy et al., 1979; Walsh et al., 1983). Furthermore, Ba^{2+} phosphorylated MLC in skinned muscle strips to almost the same extent as in the Ca^{2+}- or Ba^{2+}-induced contraction (0.3 μM Ca^{2+} vs. 0.1 mM Ba^{2+}). These results indicate that Ba^{2+}, like Ca^{2+}, produces contraction through phosphorylation of MLC as a result of the activation of MLCK by a Ba^{2+}-calmodulin complex.

Chao et al. (1984) found that Ba^{2+} neither affected the binding of $^{45}Ca^{2+}$ to calmodulin nor induced any significant change in the tyrosine fluorescence that occurred as a result of conformational changes of calmodulin, and Ba^{2+} also did not activate the phosphodiesterase up to a concentration of 0.4 mM in the presence of 10 μM calmodulin. On the other hand, Wolff et al. (1972) showed that Ba^{2+} was a competitive inhibitor of $^{45}Ca^{2+}$ binding to calmodulin in pig brain, with a low affinity. Shimizu and Hatano (1984) found that Ba^{2+} induced negative circular dichroism bands for the TFP- (90 μM) calmodulin (45 μM) complex and suggested that Ba^{2+} interacts with the TFP-calmodulin complex. In the present experiments, the Hill coefficient calculated from the pCa-tension relationship was 2, compared with ~1 from the pBa-tension relationship. The nature of this change is not clear, but since the phosphorylation induced by Ba^{2+} or Ca^{2+} corresponded closely to the amplitude of the contraction evoked by these cations, presumably the manner of formation of the Ba^{2+}-calmodulin complex and/or the activation of MLCK by this complex differs from that for the Ca^{2+}-calmodulin complex. However, it is plausible that the production of the Ba^{2+}-calmodulin complex may be responsible for the generation of the Ba^{2+}-induced contraction. Discrepancies concerning the action of Ba^{2+} on the contractile proteins obtained from the present experiments and on the phosphodiesterase activity reported by Chao et al. (1984) using biochemical procedures should be clarified by further experiments.

In conclusion, Ba^{2+} can evoke contraction in vascular smooth muscle by the following processes: Ba^{2+} does not pass the plasma membranes through the receptor-operated Ca^{2+} channel, but through the voltage-dependent Ca^{2+} channel; it releases Ca^{2+} from the intracellular store site but is not accumulated into the store site; and it directly activates the contractile proteins as a result of the activation of MLCK by the Ba^{2+}-calmodulin complex.

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